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(54) Reagents for the determination of drugs.

(57) Novel reagents for the detection by immunoassay of drugs in body fluids, their preparation and use are disclosed. The reagents of the present invention correspond to the formula

$P\text{-}[A\text{-}D]_n$

where:
D is a drug derivative suitably selective for the determination of the presence of the target drug or drug metabolite,
A is an activating linker-spacer group having an N-hydroxysuccinimide or isothiocyanate derived linking moiety,
P is a poly(amino acid) or polymer capable of covalently bonding with A, and
n is less than 1.

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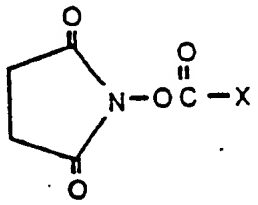
The ratio of activated drug derivative A-D to poly(amino acid) or polymer is an essential aspect of the reagents of the present invention and their high degree of sensitivity and reliability for the detection of drugs in the resulting diagnostic test. Because of their excellent antigenic reactivity, it has been found that for the activated drug derivatives of the present invention the ratio n of A-D to P must be less than 1, and typically below 0.5. Thus, allowing for the normally less than 100% yield that can be expected from the conjugation reaction of A-D with P (yields for the activated drug derivatives of the present invention with e.g. bovine serum albumin being most typically in the 50 to 75% range), the starting stoichiometric ratio of A-D to P for the reaction should be selected accordingly.

The optimal ratio n for a given activated drug derivative in accordance with the present invention can be determined readily by plotting reaction sensitivity in a desired assay format against the stoichiometric ratio of the reactants, i.e., A-D/P. Figure 1 shows a typical result of such a plotting for PCP (phencyclidine) and cocaine (benzoylecgonine) reagents in the case of a latex agglutination assay format. In accordance with standard practice, these assays are run against minimum clinically effective cutoff concentrations, i.e., as specified by the National Institute on Drug Abuse (Department of Health and Human Services), which e.g. for phencyclidine is 25 ng/ml and for cocaine is 300 ng/ml.

The activating linker-spacer group A can be a group of the formula



or S = C = N-X

where X is an optional spacing group comprising a benzene, amide, thiourea or urea group or a straight or branched aliphatic chain of C_{1-10}, or a combination of the foregoing.

The drug derivative D may be a target drug itself, its metabolite or any other derivative which is sufficiently antigenically selective so as to indicate the presence of target drug or drug metabolite in a tested sample of bodily fluid, e.g., urine.

The activating group A is covalently linked to the drug, drug metabolite or drug derivative of choice at a sterically and reactively appropriate site which in the case of drugs of abuse reagents are preferably as follows:

For amphetamine and methamphetamine: at the ortho, meta or para carbon of the benzene ring, preferably para.

For THC: at the 9 carbon of the Δ^8THC derivatives.

For barbiturate: at the 5 carbon of 5-substituted barbituric acid derivatives.

For phencyclidine: at the ortho, meta or para carbon of the benzene ring, preferably para.

For morphine: at the 3-hydroxyl.

For benzoyl ecgonine: at the meta or para carbon of the benzene ring, preferably para.

For 1,4 benzodiazepines: at the 1 nitrogen.

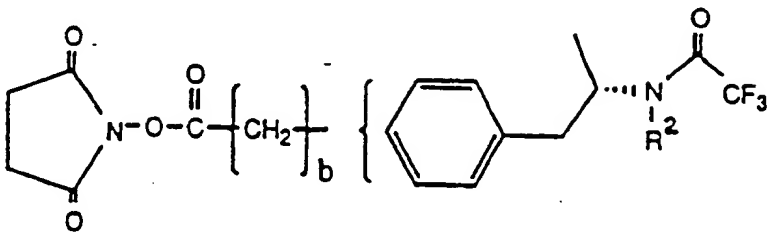
The reactive amino groups of P bind to A through amide (in the case of N-hydroxysuccinimide ester) or urea or thiourea (in the case of the isothiocyanates) linkage.

The subsequently purified protein conjugate is attached either covalently or hydrophobicly, usually covalently to a microparticle that will be used as the antigenic reagent in a specific diagnostic test for the detection of drug.

Suitable microparticles for the covalent attachment of activated drug derivative-protein conjugates would be carboxylated styrene butadienes, carboxylated polystyrenes, acrylic acid polymers and the like. Suitable micro-particles for the hydrophobic attachment of protein conjugate would be polystyrene, polyvinyltoluene, polydivinylbenzene, polyvinylchloride, polytertiary butylstyrene and the like. Suitable microparticles for the direct covalent attachment of the activated ester would be amino polystyrene, aminopolyvinyltoluene and the like. Suitable magnetic microparticles for the attachment of protein conjugates would be the respective polymer impregnated with a magnetic metal such as ferric or ferrous oxides.

The size of microparticles will typically range from about 0.01 to about 1.0 microns. The size of magnetic microparticles will range from about 0.1 to about 20 microns.

For amphetamine and methamphetamine * , a preferred activated drug derivative is the N-hydroxysuccinimide ester derivative of the formula:

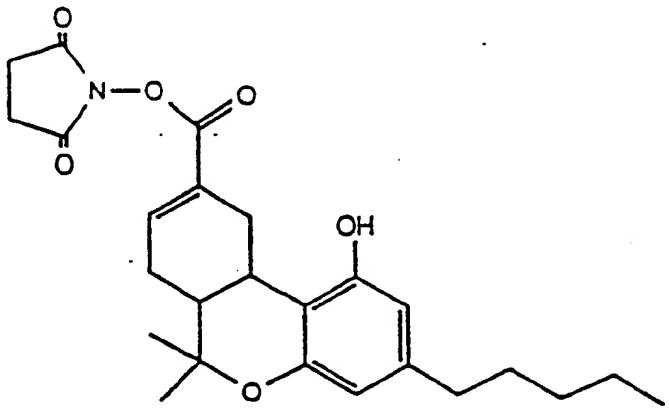


where: R^2 is hydrogen in the case of amphetamine and methyl in the case of methamphetamine, b is 1 to 9, preferably 3, and the alkyl chain is meta or para, preferably para.

The activated ester is prepared by reacting d-amphetamine (or methamphetamine) with trifluoroacetic anhydride. The resulting N-protected amphetamine is reacted with succinic anhydride in the presence of aluminum chloride to give a substituted protected amphetamine which contains an acid and ketone. The ketone is reduced with palladium on charcoal in an atmosphere of hydrogen and the acid is then converted to an activated ester with N-hydroxysuccinimide in the presence of a carbodiimide.

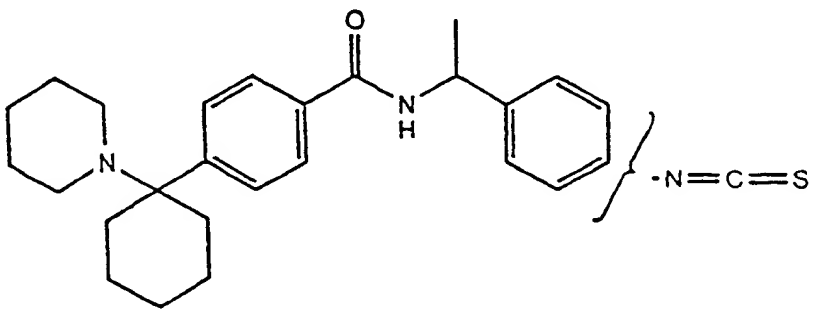
The protecting group, in this case the trifluoroacetyl, is removed prior to use in an antibody mediated assay, as for example after preparing a microparticle (e.g. latex) reagent.

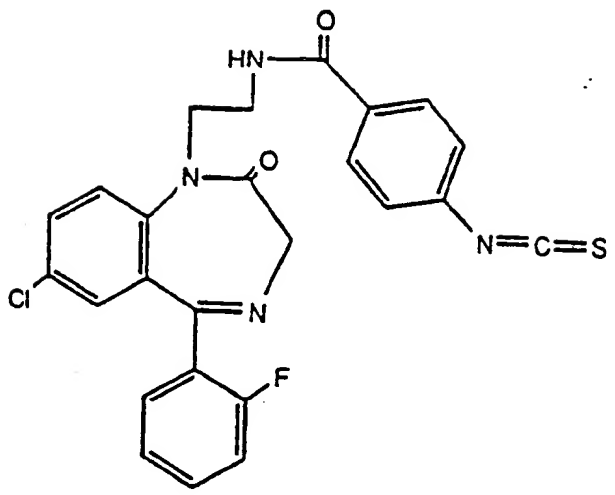
For tetrahydrocannabinoid, a preferred activated drug derivative is the N-hydroxysuccinimide ester derivative of the formula:



Such derivatives may be prepared by reacting the appropriate cannabinoid derivative, such as disclosed in J. Org. Chem. (1986), 51, pp. 5463-5465 with N-hydroxysuccinimide in the presence of a carbodiimide.

For phencyclidine (PCP), preferred activated drug derivatives are the isothiocyanate of the formula





This isothiocyanate may be prepared by reacting 1-(2-aminoethyl)-7-chloro-1,3-dihydro-5-(2-fluorophenyl)-2H-1, 4-benzodiazepin-2-one as disclosed in J. Med. Chem., (1968), 11, pp. 774-777 in the same manner as described in Example 1 below where the amino group is reacted with protected para-aminobenzoic acid and carried on to the isothiocyanate.

Of the foregoing activated drug derivatives, the following compounds are most preferred:

For morphine: N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-isothiocyanatobenzamide.

For barbiturate: 5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxo=propyl]-5-(2-propenyl)2,4,6(1H,3H,5H)-pyrimidinetrione.

For amphetamine: (S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide.

For methamphetamine: (S)-N-Methyl-N[2-[4-[4-[(2,5-dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide.

For tetrahydrocannabinoids: 1-[[(6a,7,10,10a-Tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo)oxy]-[b,d]pyran-1-yl (carbonyl]oxy]-2,5-pyrrolidinedione.

For phencyclidine: N-[1-(4-Isothiocyanophenyl)ethyl]-4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide.

For benzodiazepines: N-[2-[7-Chloro-5-(2-fluorophenyl)-2,3-dihydro-2-oxo-1H-1,4-benzodiazepin-1-yl]ethyl]-4-isothiocyanatobenzamide.

For benzoylecgonine: [1R-(exo,exo,anti)]-8-Methyl-3-[[4-[[[[[[[(2,5-dioxopyrolidin-1-yl)oxy]carbonyl]phenyl]-amino] (thioxomethyl)]amino]methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic acid.

Preferred poly(amino acid) conjugates of the above activated drug derivatives are the bovine serum albumin (BSA) conjugates and the preferred microparticle reagent embodiment incorporates these conjugates by covalent amide bonding to the surface of carboxylated latex microparticles.

The invention is further explained and illustrated in the following examples. All temperatures are in degrees centigrade.

Example 1

Preparation of opiate reagent

Preparation of N-[3-[(7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-N-(butylcarbamoyl)benzamide:

A stirred solution of 0.5g (1.46 mmol) of 3-0-amino-propylmorphine and 0.5g (2.1 mmol) of p-(1-t-

tangential flow filtration. To the washed 2% latex were added 900 ml of the opiate-BSA solution from Example 1. The reaction was stirred overnight at 4° and was again extensively washed with water by tangential flow filtration. The washed latex was then adjusted to a 3% latex solids (by weight) concentration.

Example 3

Opiate Test

Preparation of reaction buffer for test:

This diluent consists of the following aqueous solution at pH 7.5:

1. Pipes [0.20M, 1,4-piperazine bis(ethanesulfonic acid)]
2. PVP [0.50%, Polyvinylpolypyrrolidone]
3. PEG 8000 [1.50%, Poly(ethylene glycol)]
4. Sodium Chloride [10%]
5. Sodium Azide [0.1%]

Preparation of antiserum buffer for test:

Purified mouse monoclonal antibody against opiates is diluted in an appropriate buffer system. This diluent consists of the following in aqueous solution in pH 7.0:

1. Pipes (0.05M)
2. BSA (0.25%)
3. Sodium Chloride (0.15%)
4. Sodium Azide (0.15%)

Test Methodology:

Ten microliters of opiate free urine is dispensed into a mixing well of a capillary agglutinography slide followed by 50 microliters each of antiserum buffer, reaction buffer and latex (example 2). The added liquids are stirred for three seconds and the solution is moved to allow contact with the capillary. The liquid will move through the capillary and fill the viewing area at the end of the capillary in three to six minutes. The liquid in the viewing area will appear as fine floccules (agglutinated) or milky. For negative samples agglutination should be very strong with very little cloudiness.

The dilution of a particular antiserum which is chosen for the test is the one which has the highest dilution that will still produce strong agglutination in the viewing area. When various amounts of opiates are dissolved in opiate free urine in the test systems, no agglutination occurs. The amount of opiate required to inhibit the agglutination will vary from 100 nanograms per ml or greater depending on the concentration of antiserum used and the strength of antiserum produced. Thus, for the system described above a urine containing 300 nanograms of opiate per ml is sufficient to partially inhibit agglutination and a urine containing 600 nanograms of opiate per ml is sufficient to completely inhibit agglutination.

The following table reports the type of cross-reactivity that is observed in the system described above:

pyrimidinetrione (barbiturate) to Bovine Serum Albumin (BSA)

To a solution (400 ml) containing sodium bicarbonate (0.05 M), 10% dimethylsulfoxide (DMSO), and BSA (6.0g) at pH 8.5, 25° the N-hydroxysuccinimide ester (0.008g in 6.6 ml of DMSO) was added dropwise. The reaction was stirred at 4° for 15 h at which point it was transferred to dialysis tubing and dialyzed first into an aqueous sodium bicarbonate-DMSO (10%) solution (0.05M, pH 8, 4 x 10 volumes) and then into a sodium phosphate buffer (0.05M, pH 7, 7 x 10 volumes). After dialysis the protein concentration was measured and adjusted to 0.005g per ml.

Example 5

Preparation of sensitized latex containing the barbiturate-BSA conjugate general procedure.

To a stirred latex (Seradyn carboxy modified polystyrene 0.888 microns) suspension (180 ml, 10% solids), N-hydroxybenzotriazole (NHB) (0.6g in 24 ml of aqueous dimethylformamide) and 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho p-toluenesulfonate (CMC) (2.0g in 32 ml of water) were added. The suspension was stirred for 3 h at 4° then diluted to 2% latex and extensively washed with water by tangential flow filtration. To the washed 2% latex were added 900 ml of the barbiturate-BSA solution from Example 4. The reaction was stirred overnight at 4° and was again extensively washed with water by tangential flow filtration. The washed latex was then adjusted to a 3% latex solids (by weight) concentration.

Example 6

Barbiturate Test

Preparation of reaction buffer for test:

This diluent consists of the following in aqueous solution at pH 7.5:

1. Hepes [0.20M, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]
2. PVP [0.50%, Polyvinylpolypyrrolidone]
3. PEG 8000 [1.00%, Poly(ethylene glycol)]
4. Sodium Chloride [1.5%]
5. Sodium Azide (0.1%]

Preparation of antiserum for test:

Rabbit antiserum against barbiturates is diluted in an appropriate buffer system. This diluent consists of the following in aqueous solution at pH 8.0:

1. Hepes, (0.05M)
2. BSA (0.25%)
3. Sodium Chloride (0.15%)
4. Sodium Azide (0.15%)

Test Methodology:

Ten microliters of barbiturate free urine is dispensed into a mixing well of a capillary agglutinography slide followed by 50 microliters each of antiserum buffer, reaction buffer and latex (example 5). The added

^{1}H NMR ($CDCl_3$) δ 1.17 (d J = 6 Hz, 3H, CH_3), 2.78 (t, J = 5Hz, 2H, CH_2), 4.22 (m, J = 6Hz, 1H, CH), 6.00 (bs, 1H, NH), 7.06 (d, J = 6 Hz, 2H, Ar), 7.22 (m, 3H, Ar)

Preparation of (S)-4-[2-methyl-2-[(trifloroacetyl)amino]ethyl]-p-oxo-benzenebutanoic acid (2):

To a stirred solution of (1), (12.0g, 0.05 mol) in methylene chloride (210 ml) under argon, succinic anhydride (8.0g, 0.08 mol) was added. The reaction was cooled in an ice bath and then treated with aluminum chloride (28.0g, 0.21 mol), portionwise over 5 min. The reaction was stirred at 0-5° for 2 h and then at room temperature overnight. Hydrochloric acid (3N, 120 ml) was then slowly added and then solution was stirred for an additional one hour. The methylene chloride was removed in vacuo and the aqueous layer was extracted with ethyl acetate. The organic layer was dried (Na_2SO_4), filtered and the solvents evaporated to yield a tan residue which upon trituration with ether gave 10.5g of product.
IR($CHCl_3$) 3300(NH), 1700 (Acid + Amide), 1555 (Amide) cm^{-1}, ^{1}H NMR($CDCl_3$ + DMSO-d_6) δ 1.21-(d,J = 7Hz, 3H, CH_3), 2.73 (t, J = 6Hz, 2H, CH_2), 2.82(m, 1H, H of CH_2), 2.95(m, 1H, H of CH_2), 3.27 (t, J = 6Hz, 2H, CH_2), 4.25(1H, CH), 7.27(d, J = 8 Hz, 2H, Ar), 7.92(d, J8Hz, 2H, Ar), 7.57(bs, 1H, NH), Ms, m/e 331(M+)

Preparation of (S)-4-[2-methyl-2-[trifloroacetyl)amino]ethyl]benzenebutanoic acid (3):

A mixture of 2 (9.2g, 0.027 mol), and 10% palladium on charcoal (4.0g) in acetic acid (400 ml) was hydrogenated at 50 psi for 24 h. The catalyst was filtered off, the filtrate was concentrated in vacuo and the residue was triturated with ether to yield 7.0g of a white product.
IR($CHCl_3$) 3425(NH), 1718(Acid), 1532(Amide), 1170(CF_3) cm_{-1}, ^{1}H NMR($CDCl_3$) δ 1.16(d, J = 7Hz, 3H, CH_3), 1.92(m, 2H, CH_2), 2.34(bs, 2H, AR-CH_2), 2.63 (m, 2H, CH_2), 2.79(m,2H,CH_2), 4.28(m, 1H, CH), 5.94-(bs, 1H, NH), 6.97(d, J = 8Hz, 2H, AR), 7.03(d, J = 8Hz, 2H, AR). MS, m/e 299(M-H_2O)

Preparation of (S)-N-[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-4-oxo = butyl]phenyl]-1-methyl-ethyl]-2-trifloroacetamide (4)

To a stirred solution of 3 (5.4g, 0.019 mol) in methylene chloride (150 ml), tetrahydrofuran (150 ml) and dimethylforamide (50 ml), N-hydroxysuccinimide (2.7g, 0.023 mol) and 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride (6.0g, 0.031 mol) was added. The reaction was stirred at room temperature overnight, and then was concentrated in vacuo. The resulting residue was dissolved in methylene chloride, filtered and purified by silica gel chromatography (7% ether-methylene chloride as eluent) to give a yellow oil which crystallized in a ether to yield 3.7g of a white product.
IR($CHCl_3$), 3425(NH), 1812-1741(Imide), 1725(Amide), 1170(CF_3) cm^{-1}. ^{1}H NMR($CDCl_3$) δ 1.21(d, J = 7Hz, 3H, CH_3), 2.07(m, 2H, CH_2), 2.60(t, J = 7Hz, 2H, CH_2), 2.72 (t, J = 7Hz, 2H, CH_2), 2.80 -2.90(m, 6H, CH_2), 4.27(m, 1H, CH), 6.10(bs, 1H, NH), 7.09(d, J = 8Hz, 2H, Ar), 7.15(d, J = 8 Hz, 2H, Ar).
MS, (+)FAB, m/e 415(M + H), m/e 437(M + Na).
Anal. Calc. for C, 55.07; H, 5.11; N, 6.76
Anal. found for C, 54.94; H, 5.22; N, 6.76

Conjugation of (S)-N-[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-4-oxobutyl]phenyl]-1-methyl-ethyl]-2-trifloroacetamide (amphetamine) to Bovine Serum Albumin (BSA):

To a solution (400 ml) containing sodium bicarbonate (0.05 M), 10% dimethylsulfoxide (DMSO), and BSA (6.0g) at pH 8.0, 25° the N-hydroxysuccinimide ester (4) (0.035g in 32.6 ml of DMSO) was added dropwise. The reaction was stirred at 4° for 15 h and dialyzed as in Example 4. After dialysis the protein concentration was measured and adjusted to 0.0005g per ml.

Example 8

inhibit agglutination.

The following table reports the type of cross-reactivity that is observed in the system described above:

	Concentration (ng/ml) equivalent to 300 ng/ml of Morphine	% Cross reactivity
p-Hydroxyamphetamine	5,000	20
Tyramine HCl	100,000	0.01

The following table reports the type of specificity and accuracy that is observed with the above described assay when used with samples positive for amphetamine:

Gas Chromatography-Mass Spectroscopy +		
Agglutination Test	+	45
	-	1*

* The one discrepant sample was shown to contain drug at a concentration close to the cut-off (1,000 ng/ml) of the assay.

Example 10

Preparation of tetrahydrocannabinoid reagent

Preparation of 1-[[(6a, 7, 10, 10a-Tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo[b,d]pyran-1-yl-(carbonyl] oxy]-2,5-pyrrolidinedione:

To a solution of 1.26g (3.67 m mole) of 11-nor-Δ^8-THC-9-carboxylic acid[1] in 50 ml of 1:1 ethylacetate/methylene chloride were added 1.68g (14.7 mmole) of N-hydroxysuccinimide and 2.10g (11.0 mmole) of 1-(3-dimethylaminopropyl)3-ethylcarbodiimide hydrochloride. The reaction was stirred for 16 hours at room temperature. The reaction mixture was washed with 1N hydrochloric acid (3 x 100 ml), water (3 x 100 ml) and saturated brine (100 ml). The organic phase was dried over Na_2SO_4, concentrated under reduced pressure and chromatographed over silica gel. Eluting with 40% ethylacetate in hexane afforded 1.41g (87%) of the desired N-hydroxysuccinimide ester. Satisfactory NMR, IR and MS were obtained. IR(KBr) 3415(OH), 1802, 1760, 1738 (c=0) cm^{-1}, ^1H NMR ($CDCl_3$)δ 0.88(t, J=6Hz, 3H, CH_3), 1.13(s, 3H, CH_3), 1.40 (s, 3H, CH_3), 2.86(bs, 4H, CH_2), 6.11(s, 1H, Ar), 6.27 (s, 1H, Ar), 7.32(d, J=4Hz, 1H, vinyl). MS, m/e 441(M+)

Conjugation of 1-[[(6a, 7, 10, 10a-Tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo)oxy][b,d]pyran-1-yl(carbonyl] oxy]-2,5-pyrrolidinedione (cannabinoid) to Bovine Serum Albumin (BSA):

To a solution (300 ml) containing sodium bicarbonate (0.05 M), 10% dimethylsulfoxide (DMSO), and BSA (4.5g) at pH 8.5, 25° the N-hydroxysuccinimide ester (0.006g in 6.2 ml of DMSO) was added dropwise. The reaction was stirred at 4° for 15 h and dialyzed as in Example 4. After dialysis the protein

agglutination and a urine containing 600 nanograms of cannabinoid per ml is sufficient to completely inhibit agglutination.

The following table reports the type of cross-reactivity that is observed in the system described above:

	Concentration (ng/ml) equivalent to 300 ng/ml of Δ^8THC	% Cross reactivity
11-Hydroxy-cannabinol	2,000	5.0
Cannabinol	800	12.5
Δ^9-THC	10,000	1.0
11-Hydroxy-Δ^9-THC	2,000	5.0
8β-11-Dihydroxy-Δ^9-THC	4,000	2.5
8β-Hydroxy-Δ^9-THC	4,000	2.5

The following table reports the type of specificity and accuracy that is observed with the above described assay when used with samples positive for cannabinoids:

Gas Chromatography-Mass Spectroscopy +		
Agglutination Test	+	43
	-	2*

* The two discrepant samples were shown to contain drug near the cut-off (100 ng/ml) of the assay.

Claims

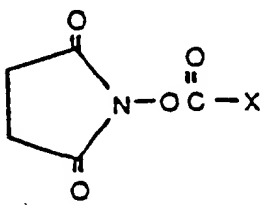
1. A reagent of the formula

$P\text{-}[A\text{-}D]_n$

where:

D is a drug derivative antigenically selective for the determination of the presence of target drug or target drug metabolite,

A is an activating linker-spacer group of the formula

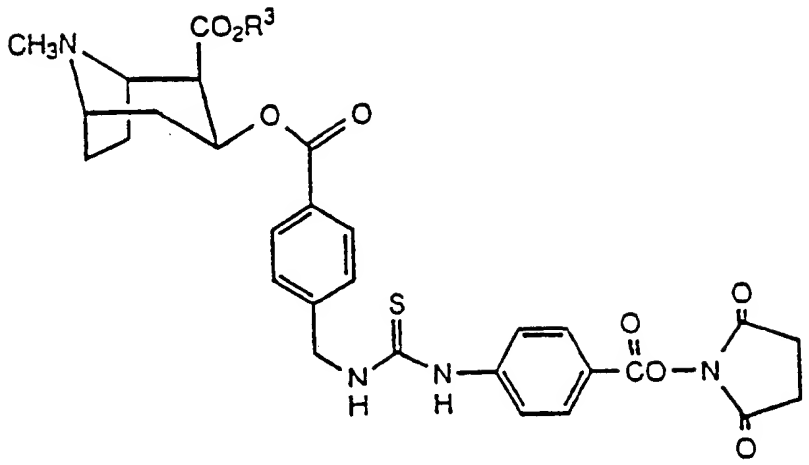


or S = C = N-X

where X is an optional spacing group linking A to D which comprises a benzene group, an amide group, a thiourea group, a urea group, a straight or branched C_{1-10} aliphatic group, or a combination of the foregoing groups,

P is a poly(amino acid) or polymer capable of covalently bonding with A, and

n is less than 1.



and R^3 is hydrogen or methyl.

7. The reagent according to claim 2, wherein A-D is a compound selected from the group consisting of:
N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-isothiocyanatobenzamide,
5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxo propyl]-5-(2-propenyl)2,4,6(1H,3H,5H)pyrimidimetrione,
(S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,
(S)-N-Methyl-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,
N-[1-(4-Isothiocyanophenyl)ethyl]-4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide,
N-[2-[7-Chloro-5-(2-fluorophenyl)-2,3-dihydro-2-oxo-1H-1,4-benzodiazepin-1-yl]ethyl]-4-isothiocyanatobenzamide,
[1R-(exo,exo,anti)]-8-Methyl-3-[[4-[[[[[[[(2,5-dioxopyrolidin-1-yl)oxy]carbonyl]phenyl]amino](thioxomethyl)-]amino] methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic acid,
1[4-[1-(1-Piperidinyl)-1-cyclohexyl]benzoyloxy]-2,5-pyrrolidinedione, and
1-[[(6a,7,10,10a-Tetrahydro-1-hydroxy-6,6-dimethyl-3-pentyl-6H-dibenzo)oxy][b,d]pyran-1-yl(carbonyl]oxy]-2,5-pyrrolidinedione.

8. A microparticular immunoassay reagent comprising the reagent of claim 1, 2 or 7 bound to microparticles by the hydrophobic or covalent bonding of the poly(amino acid) or polymer, P, to the surface of said microparticles.

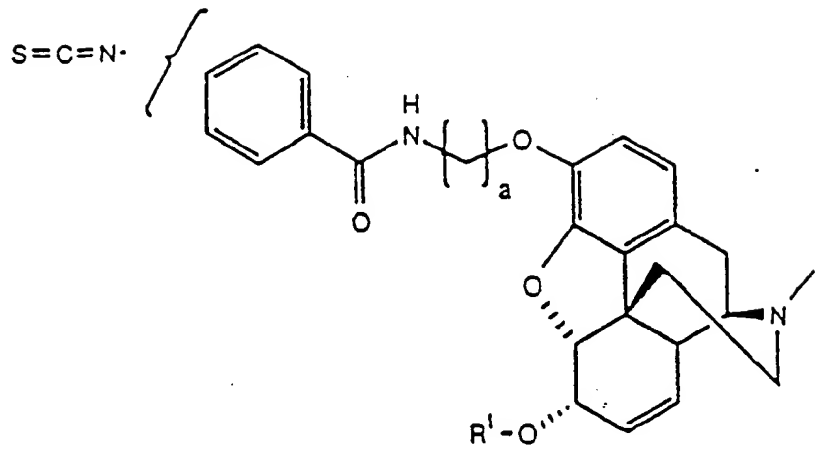
9. The microparticular reagent of claim 8, wherein P is covalently bound to latex microparticles.

10. The reagent of claim 9, wherein P is bovine serum albumin.

11. A compound selected from the group consisting of:
N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-isothiocyanatobenzamide,
5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxo-propyl]-5-(2-propenyl)2,4,6(1H,3H,5H)pyrimidimetrione,
(S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,
(S)-N-Methyl-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,
N-[1-(4-Isothiocyanophenyl)ethyl]-4-[1-(1-piperidinyl)-1-cyclohexyl]benzamide,
N-[2-[7-Chloro-5-(2-fluorophenyl)-2,3-dihydro-2-oxo-1H-1,4-benzodiazepin-1-yl]ethyl]-4-isothiocyanatobenzamide,
[1R-(exo,exo,anti)]-8-Methyl-3-[[4-[[[[[[[(2,5-dioxopyrolidin-1-yl)oxy]carbonyl]phenyl]amino](thioxomethyl)-]amino]methyl]benzoyl]oxy]bicyclo[3,2,1]octane-2-carboxylic acid, and
1-[4-[1-(1-Piperidinyl)-1-cyclohexyl]benzoyloxy]-2,5-pyrrolidinedione.

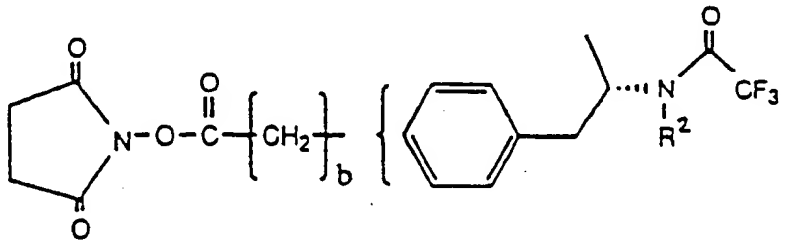
Claims for the following Contracting State: ES

1. A process for the detection of drug use by immunoassay testing of bodily fluids, characterized in that a reagent of the formula



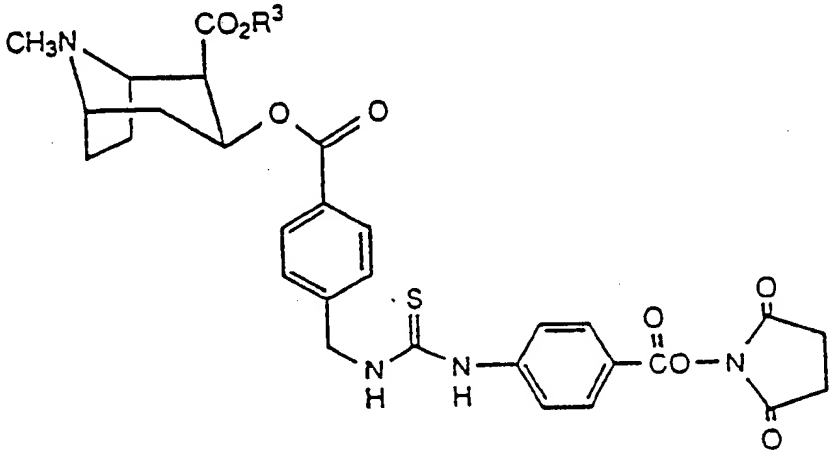
where R_1 is hydrogen, methyl or acetyl, a is 2 to 8, and the isothiocyanate is meta or para.

5. A process as claimed in claim 2 wherein A-D is a compound of the formula:



where R^2 is hydrogen or methyl, b is 1 to 9, and the alkyl group is bound to the benzene ring in the meta or para-position.

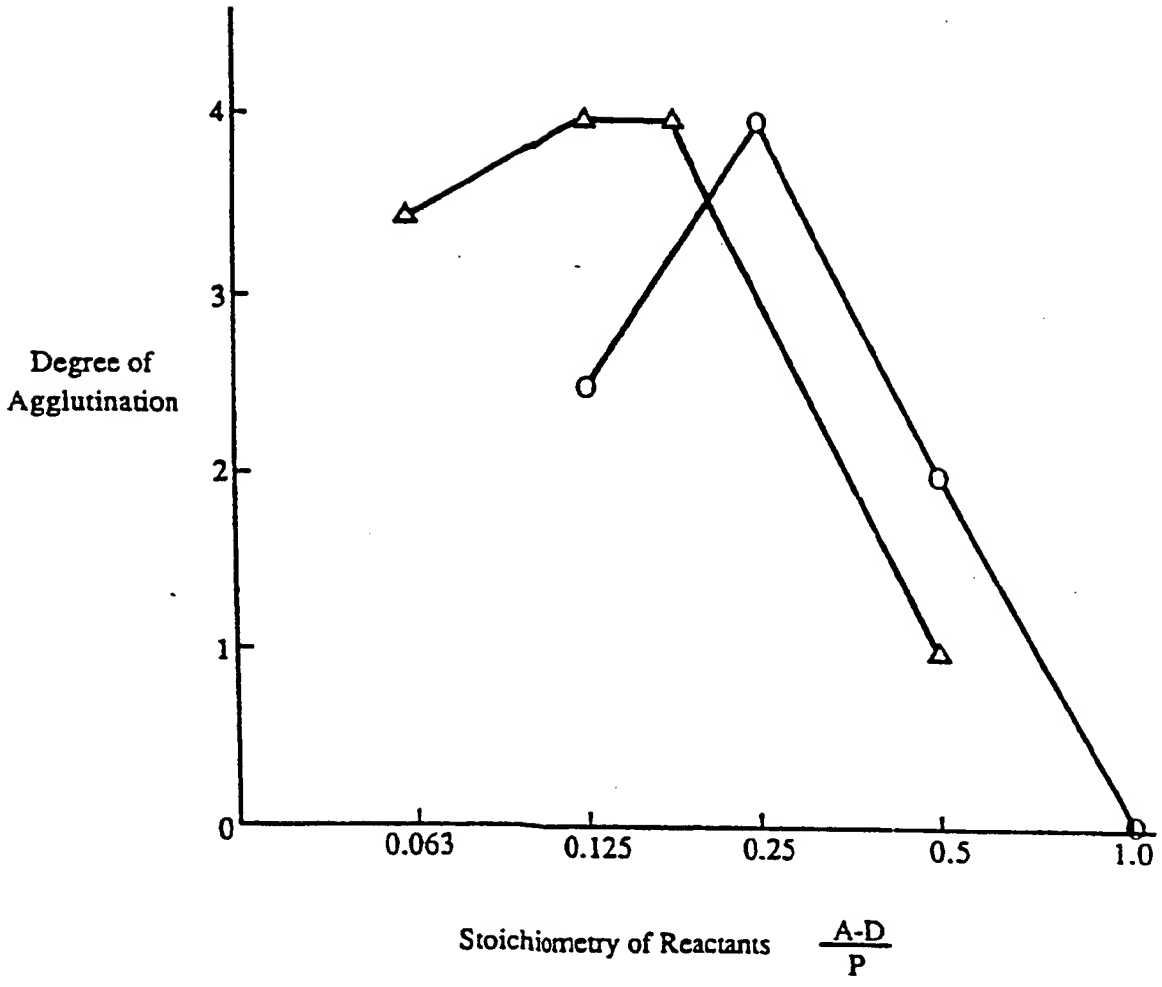
6. A process as claimed in claim 2 wherein A-D is a compound of the formula:



and R^3 is hydrogen or methyl.

7. A process as claimed in claim 2, wherein A-D is a compound selected from the group consisting of:
N-[3-[7,8-Didehydro-4,5-epoxy-6-hydroxy-17-methylmorphinan-3-yl)oxy]propoxy]-4-isothiocyanatobenzamide,
5-[3-[2,5-Dioxo-1-pyrrolidinyl)oxy]-1-methyl-3-oxo-propyl]-5-(2-propenyl)2,4,6(1H,3H,5H)pyrimidimetrione,
(S)-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-trifluoroacetamide,
(S)-N-Methyl-N[2-[4-[4-[(2,5-Dioxo-1-pyrrolidinyl)-oxy]-4-oxo-butyl]phenyl]-1-methyl-ethyl]-2-

FIGURE 1



O = Benzoylecgonine Reagent

Δ = Phencyclidine Reagent

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(54) Reagents for the determination of drugs.

(57) Novel reagents for the detection by immunoassay of drugs in body fluids, their preparation and use are disclosed. The reagents of the present invention correspond to the formula

$P-[A-D]_n$

where:

D is a drug derivative suitably selective for the determination of the presence of the target drug or drug metabolite,

A is an activating linker-spacer group having an N-hydroxysuccinimide or isothiocyanate derived linking moiety,

P is a poly(amino acid) or polymer capable of covalently bonding with A, and

n is less than 1.

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CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

☐ All claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid,

namely claims:

☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

☒ LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirement of unity of invention and relates to several inventions or groups of inventions,

namely:

1. Claim 1: in as far as A is 2,5-dioxo-1-pyrrolidinyl-oxo-carbonyl-X; claims 2,7-10: in as far as D is a Δ^8 THC derivative;

2. Claim 1: in as far as A is thiocyanato-X; claims 2,8-10: in as far as D is a Δ^8 THC derivative;

3. Claim 1: in as far as A is 2,5-dioxo-etc; claims 2,7-11: in as far as D is a barbituric acid derivative;

4. Claim 1: in as far as A is thiocyanato-X; claims 2,8-10: in as far as D is a barbituric acid derivative;

5. Claim 1: in as far as A is 2,5-dioxo-etc; claims 2,7-11: in as far as D is a phencyclidine derivative;

... / ...

☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.

☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid.

namely claims:

☒ None of the further search fees has been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims.

1 (part),2,7-10 (part)

namely claims: